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(54) Title: PROCESS FOR PREPARING OIL COMPOSITION			
(57) Abstract The invention provides a process for preparing oil compositions from a group of fishes consisting of Hypophthalmichthys and Abramis species, the said compositions being useful for completing alimentation in order to prevent pathological consequences of arteriosclerosis. The process according to the invention uses the fatty tissues from the abdominal cavity of the above species as starting material. The compositions thus prepared have a very high content on omega-3 fatty acids.			

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## PROCESS FOR PREPARING OIL COMPOSITION

Technical field

The present invention relates to the preparation of oil compositions with high omega-3 unsaturated fatty acids from Hypophthalmichthys and Abramis species.

5 Background-art

Arteriosclerosis and its consequences, such as myocardial infarction caused by coronary occlusion are one of the most frequent cause of death in advanced countries. The increasing frequency thereof is generally attributed to the characteristic change in the mode of  
10 life and alimentation, which increase the liability and hazards by known way.

In alimentation substantial modification and distortion in the quantity and composition of fatty materials can be observed when comparing the diet of people living in natural environment with dietary  
15 habits of those living in big cities. A well known consequence of this change in the composition is hyperlipidaemia, the increased cholesterol level of blood. To prevent arteriosclerosis, screening controls have been introduced in many countries.

In the last decade it has been found that the balanced distribution of unsaturated fatty acid in the food has a very important role.  
20 These fatty acids are precursors of a family of compounds metabolized in the organism, the said compounds having important regulation function. If the food lacks of a certain group of unsaturated fatty acids, the functional equilibrium of the regulating compounds thus obtained be-

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comes unbalanced leading to serious consequence in the health.

The above phenomenon was supported by the fact that substantially no arteriosclerosis, hypertension and thrombosis could be detected at the Eskimos and in certain Japanese fisher villages.

- 5 Genetical causes could be excluded and the answer to the difference was explained by the very characteristic alimentation rich in fish. The annual fish consumption of Eskimos takes several hundred kilograms per person while the same value is 7 kg per year in the USA and 3 kg per year in Hungary. The variety of these fishes is also very important: they are plant eating cold-sea fishes which are rich in fats and the fat composition is delicate due to the omega-3 unsaturated fatty acid content thereof [Am. J. Clin. Nutr., 28, 958 /1975/, Lancet 1, 117 /1978/, Lancet 2, 433 /1979/].
- 10

- The fatty acids occur in the organism in the form of phospholipids, triglycerides and free fatty acids. According to their origin and function they can be distributed in four main groups:
- 15

- Saturated fatty acids:

- They are metabolized from any carbon source by any organism through acetic acid. They are present in the human food mostly as animal fat.
- 20

- Omega-9 unsaturated fatty acids:

Characteristic and starting material of this group is the oleic acid. They are synthesized by animal and plant organisms.

- Omega-6 unsaturated fatty acids:

- Characteristic and starting material of this group is the linoleic acid. They are present in plant tissues, in the first line in seeds.
- 25

- Omega-3 unsaturated fatty acids:

- The characteristic and starting material of this group is linolenic acid. They are synthesized by microorganisms living in the
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water and deposited in the tissues of fishes feeding on the said organisms. As human food they can be taken up only by eating fishes but get into the organism in very small quantities only. The importance thereof is proved by the fact that this small amount mostly occurs in the brain tissues. They are non-essential compounds but in the absence thereof regulation disturbances and illnesses might occur and the deficiency thereof indirectly and together with other factors may cause even death.

The fatty acids are labelled as X:Y omega Z/number of carbon atoms: number of double bonds and Z means the length of the saturated carbon chain after the last double bond including the terminal methyl group/.

The above mentioned fatty acids are labelled as follows:

- 18:1 omega-9 = oleic acid;
- 18:2 omega-6 = linoleic acid;
- 18:3 omega-3 = linolenic acid.

The group of  $C_{20}$  fatty acids is that of eicosaic acids including the 20:4 omega-6 arachidonic acid /AA/ and 20:5 omega-3 eicosapentaenoic acid /EPA/.

The group of  $C_{22}$  fatty acids is that of docosaic acids including among others the 22:1 omega-9 erucic acid and 22:6 omega-3 docosahexaenoic acid /DHA/.

In the formation of the above regulating materials the  $C_{20}$  eicosaic acids play an important role and the group of  $C_{20}$  compounds thus obtained are named as eicosanoides. Functional derivatives obtained therefrom are the thromboxanes formed by ring closure /Proc. Natl. Acad. Sci., 1975, Hamberg/ and the prostaglandines. The latter group has already previously been known but only recently discovered that they are derived from the eicosaic acid /Nature, 1976, Moncada/. The leukotrienes are also obtained through additional

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metabolic routes /Proc. Natl. Acad. Sci., 1979, Murphy/ and even nowadays further metabolites and functions have become known.

5 The biosynthetic route of eicosanoids is illustrated by Fig. 1. The natural starting material thereof is the arachidonic acid /AA/ which can be found in the phospholipids. During ring-closure two double bounds will be cleaved and delta-5, delta-14-dienes are formed. Accordingly, this group is also named as "diene metabolites".

10 The functions of the metabolites illustrated on Fig. 1 are as follows:

PGH<sub>2</sub> : vasoconstrictor, ADP-releasing, blood platelet activating. The adhesion of active blood platelets increases and a hormone will be produced which stimulates the proliferation of vessel wall cells. They also promote the release of serotonin and the production of thromboxane.

PXA<sub>2</sub> : aggregates blood platelets, promotes thrombus formation.

20 LTB<sub>4</sub> : induces inflammations, stimulates the immune reactions.

12 HETE : chemotactic effect on leukocytes.

PGL<sub>2</sub> : vasodilator.

TXB<sub>2</sub> and 6-keto-PGF<sub>1</sub> : byproducts which are secreted from the organism.

25 Accordingly, the system of eicosanoides responses to the damaging influences, such as penetration and deposit of foreign materials, vessel wall lesions, etc. with inflammation, allergic reactions, thickening of vessel wall and vasoconstriction, as well as blood clogging at the area of lesion.

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The basic principle of each regulation that the responses on the effect should be repressed in certain extent, otherwise the activated regulation system turns the conditions into the opposite, also non-desired direction.

5           In the present case the organism responses so that the eicosapentaenic acid /EPA/ and derivatives thereof are analogous substrates of the same enzyme systems. These molecules compete with the corresponding members of AA metabolites and push the former off the surface of the enzymes while their own metabolites are practically  
10           ineffective.

          The two starting materials and metabolites thereof differ from each other in the delta-17 double bond. The metabolites of EPA origin are called as "triene" metabolites. Accordingly, the balance of organism depends on the equilibrium of the diene vs. triene  
15           metabolites /Nature 307, 165, 1984/.

          The biosynthetic routes which can be derived from EPA are shown in Fig. 2.

          The function of metabolites shown in Fig. 2 are as follows:

PGH<sub>3</sub> : slightly vasodilating.  
20           TXA<sub>3</sub> and LTB<sub>5</sub> : practically ineffective.  
          PGI<sub>3</sub> : substantially as vasodilating as PGI<sub>2</sub>.

          The triene metabolites increase the cyclic AMP level which inhibits phospholipase A<sub>2</sub> and, accordingly, the formation of AA.

          The 22:6 omega-3 docosaheptaenic acid /DHA/ has an effect  
25           identical to that of EPA. The omega-3 acids having lower carbon atom number are transformed into EPA in the organism by the so-called "elongation process". The metabolic passway and metabolite functions are described e. g. in the following literatures: Proc. Natl. Acad. Sci., 76, 944 /1979/; B. B. A., 875, 369 /1986/; New England j.  
30           ci Med., 314, 937 /1986/; Thrombosis Res., 42, 99 /1986/;

Nutr. Reviews, 44, 205 /1986/.

Biochemical tests, animal and clinical experiences proved that a permanently high omega-6 to omega-3 ratio in the distribution of unsaturated fatty acids might increase the susceptibility to vasoconstriction, thrombosis, allergy and inflammations. By correcting the said ratio, the diseases can be prevented and the symptoms of illnesses can be suppressed, respectively. To correct the ratio, administration of omega-3 unsaturated fatty acids would be necessary under the present general alimentation habits. The fish meat or composition rich in omega-3 unsaturated fatty acids favourably influences the lipid content of blood plasma, the so-called VLDL and LDL fractions decrease against the HLD fraction which symptom reduces the deposition of cholesterol and promotes secretion [Prog. Lipid. Res., 25, 461 /1986 /]. According to other experiments the corrected omega-6 to omega-3 ratio is regarded as an important factor in the treatment of certain skin diseases [Arch. Dermatol. 122, 1277 /1986 /]. Moreover, research in the field of malign proliferation and methastases also proved the role of deformed fatty acid ratio [Prog. Lipid. Res., 25, 583 /1986/].

The aforesaid discoveries strongly promoted research and the campaigns for increasing fish, in the first line cold sea fishes, such as sardine, mackerel, salmon, codfish, herring consumption. It has also been shown that also some fresh-water fishes can be sources for omega-3 unsaturated fatty acids. The species in question was *Cyprinus carpio* with a fat content of 7 %, wherein the EPA-content is 4,0 % of the total fat quantity and the AA-value is 1,3 % [Lancet, 717, 1983 and Prog. Lipid. Res., 25, 207 /1986/].

The article of *Aquaculture Hungarica* 1, 35 /1978/ reports on examinations for the fatty acid content of *Hypophthalmichthys* species which widely bred in Hungary. The article reports on the influence of feeding. According to the results the EPA-content in the said species may amount to 8,3 or even 9,9 % of the total fat content. The corresponding AA-values were found as 7,5 and 8,5 %, respectively.

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According to alimentation experts, the extensive fish consumption might cause pesticide poisoning as the said chemicals are deposited in fishes in the first line. For this reason, and to ensure a reproducible administration the production and sale of compositions containing omega-3 unsaturated fatty acids was first introduced in the USA. These are most frequently capsulated oils with a content of 20 to 50 % of omega-3 unsaturated fatty acids /EPA and DHA/.

These possibilities, however, did not have basic influence on correcting the alimentation. As well-known, prolonged consumption of certain fatty acids is harmful to the organism. Accordingly, C<sub>20</sub> and C<sub>22</sub> monoene fatty acids are characteristically deposited in the heart muscle membranes, thus causing a progressive degeneration and impulse conduction disturbances, i. e. pathological EKG.

The C<sub>22</sub> erucaic acid can be found in rape oil in greater quantity. Even nowadays great efforts are being made to improve rape free from erucaic acid [Lipids, 746 and 548 /1972/].

The oil compositions available in 1986 were not analyzed in this respect. Our examinations provided unfavourable results, some examples thereof are illustrated herebelow:

	EPA	DHA	20:1 and 22:1
cod liver	10 %	11 %	24 %
SUPER-EPA	30 %	20 %	11 %
LOVITRON	9 %	11 %	20 %

Also the additional compositions, like PROMEGA, PROTOCHOL or OMEGA-3-EPA contain 55 to 86 % mixed ballast material beside the active material.

The present invention aims to provide a solution to eliminate the above harmful effects.

Disclosure of invention

- It has been found that the above aim which is very important for both the common health and medical treatment can be fulfilled without holding additional effects harmful to the organism. It has been found that a preparation with high content of omega-3 unsaturated fatty acid and free from 20:1 and 22:1 fatty acids can be prepared by using intra-abdominal fat of wild or bred *Hypophthalmichthis* species: silver carp [H. molitry /Valucianne/] and big head carp [H. nolis /Richardson/] and certain bream /Abramis/ species, preferably Abramis brama, respectively, as starting material. The lipids are extracted therefrom with organic solvent, solvent mixture, hot water or steam and the lipid mixture thus obtained is enriched in fatty acid of higher unsaturation by forming lipid-urea complexes and separating the same.
- The tissues of *Hypophthalmichthis* and *Abramis* species, respectively, are characterized by essentially not containing 20:1 and 22:1 fatty acids and by a relatively high EPA- and DHA-content. The fat content of muscles is low /3%, i. e. about one tenth of that of the sea fishes listed hereabove but a substantial quantity of fat deposit can be found on the viscera which have previously been treated as debris. The fatty tissue separated from the intestines and omentum, besides a fat content of almost 100 %, surprisingly and preferably shows a very low AA-content. The main part of fatty acids consists of saturated fatty acids and oleic acid, the amount of which may be decreased substantially by urea-complex forming and thus the active ingredient content /EPA, DHA and omega-3 fatty acid transformed to C<sub>18</sub> polyene EPA/ can be increased up to 60 %.

- The purification by forming urea complex is well known from the lipid literature [H. P. Kaufman: Analyse der Fette und Fettprodukte, Band I, p. 76, Springer Verlag, /1958/. The method is based on the recognition that monoene or saturated fatty acids form

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complex with urea while fatty acids of higher unsaturation cannot close urea in complex. The said complex precipitates from the system. This method is used for treating sea fish oils by US-PS No. 4,377,526. By this method EPA of fine chemicals purity /75 to 93 %/ is obtained, wherein the urea concentration is performed as the first or second step. The separation methods of the single components include fractional distillation, fractional crystallization at low temperature, molecular distillation or ion-exchange chromatography on silver-saturated resin.

The process according to the present invention possesses the following advantages:

- the Hypophthalmichthys species are well increasable and simple fish variety,
- by using the fatty tissue the quantity to be extracted decreases to one tenth,
- by using the fatty tissue hardly disposable waste material can be utilized, i. e. a material detrimental to environment is used,
- the characteristic lipid composition of the starting material ensures to obtain a composition with high active ingredient content and free of harmful material by using simple, one-step purification,
- the bream /Abramis/ species can be fished also industrially and no specific breeding is necessary.

The compositions obtained according to the present invention serve for food completion and health care. As such, it is superior due to the high active ingredient content /EPA + DHA/ and preferably low AA-content thereof and is harmful as even traces of 20:1 and 22:1 acids cannot be found therein.

A further advantage of the process according to the present invention is that the lipid extraction is carried out on valueless waste

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material, the quantity of which is substantially less than that of the flesh or whole fish. The crude product obtained can effectively be purified by a simple single step.

The compositions prepared according to the present invention may be used by mixing in the food or capsulated against vasoconstriction, arteriosclerosis, vascular obstruction, allergic dermatitis and for preventing tumorous methastases. The compositions can also be used for adjusting the correct diet in case of the aforesaid illnesses. The daily doses can vary between 0,2 to 2,0 g, depending on the indication and duration of treatment.

#### Brief description of the drawings

Fig. 1 shows the biosynthetic route of eicosanoids.

Fig. 2 shows the biosynthetic routes starting from EPA.

Fig. 3 shows the fatty acid composition of the starting oil

in Example 9.

Fig. 4 shows the analysis of the fatty acid mixture obtained in Example 9.

#### Best mode of carrying out the invention

##### Example 1

In processing silver carp 3 years old /weighing 1,2 kg bred in pond/ the inner parts were removed and the thick fatty tissue deposited on the viscera was manually separated. The amount of tissue obtained from one animal averaged 135 g. From the fat tissue and flesh each 1 g samples were taken and extracted three times with each 5 ml of petroleum ether so that the tissue was homogenized in the presence of 2 g of dry sodium sulfate with the first charge of solvent, the solid parts were filtered off, and suspended on the filter twice with each 5 ml of petroleum ether. The three solvent fractions were combined and distilled in vacuo until oily residue was obtained. The oil was subjected to gas chromatography.

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The result obtained were compared with those obtained from the extract of carp flesh.

	Fatty acid	Silver carp		Carp
		Fat tissue from abdominal cavity	Flesh	Flesh
5	14:0	3,0	1,7	1,6
	16:0	16,1	18,3	17,0
	18:0	0,4	4,6	5,0
	16:9 omega-9	7,9	5,2	7,0
10	18:1	40,4	12,6	54,7
	20:1	-	-	1,8
	22:1	-	-	-
	18:2 omega-6	1,5	2,6	6,3
	20:2	1,5	0,1	-
15	20:3	-	0,4	-
	20:4	0,5	8,8	1,9
	22:4	0,1	0,9	0,2
	22:5	0,5	3,3	0,2
	18:3 omega-3	1,4	4,3	0,7
20	18:4	nyom	0,2	-
	20:4	-	-	-
	20:5	4,6	12,4	0,7
	22:5	0,7	2,4	0,1
	22:6	3,8	12,3	0,6

25

The fat from the abdominal cavity did not contain 20:1 and 22:1 fatty acids and had a very low AA-content. Though the EPA- and DHA-content was relatively low compared with that of the flesh, but in

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relation to the total weight of the tissue to be extracted the effective EPA- and DHA-content of the flesh was about 1/10 of that of the fat tissue.

### Example 2

- 5            Processing of big head carp, 3 years old, weighing 2100 g:  
 After dissecting and gilling, the fat tissue on the viscera was manually separated and thus 200 g of tissue was obtained. Each 1 g of samples  
 were taken from the fat tissue and the flesh of fish and extracted and  
 analyzed by gas chromatography as described in example 1. The results  
 10        obtained were compared with that of the oil pressed from the whole  
 mackerel.

	Fatty acid	Big head carp		Mackerel
		Fat tissue from abdominal cavity	Flesh	Flesh
15	14:0	4,2	2,0	6,0
	16:0	20,1	17,4	15,1
	18:0	2,4	6,1	2,3
	16:1 omega-9	13,5	3,4	3,9
	18:1	31,5	10,0	13,8
20	20:1	1,1	0,9	8,4
	22:1	-	-	10,5
	18:2 omega-6	1,7	2,8	1,1
	20:2	0,2	-	0,2
	20:3	0,2	0,3	-
25	20:4	1,0	8,9	0,8
	22:4	-	0,6	-
	22:5	0,1	2,3	-

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/cont. /

	18:3 omega-3	1,9	3,8	1,4
	18:4	-	0,2	3,3
	20:4	-	0,7	0,3
5	20:5	6,6	14,3	7,0
	22:5	1,4	2,4	0,6
	22:6	2,4	16,7	10,5

-----

The fatty acid composition of the fat tissue from the abdominal cavity of big head carp was similar to that of the silver carp, the fat composition of the flesh altered in a higher AA-content and the percentage of EPA- and DHA-content. However, in case of using the flesh for the oil extraction the weight of material to be extracted was again multiple. In case of mackerel, though it constitutes a good source of EPA and DHA, the high 20:1 and 22:1 content of the product could be detected.

### Example 3

100 g of fatty tissue obtained according to example 1 were homogenized with 2000 g of a mixture of chloroform and methanol  $\sqrt{2}:1$ , v/v by using high speed laboratory homogenizer, e. g. MSE homogenizer, for 2 x 3 minutes at n = 8000 rpm. The emulsion thus obtained was allowed to stand for 3 hours, then the proteins forming the precipitate were removed by filtering on Whatman 1PS paper and to the filtrate 400 ml of 0,2 M aqueous potassium chloride solution was added. The system was allowed to separate into two layers and after completion of the separation the lower phase containing the lipids was separated and the upper phase discarded. The chloroform solution was dried over dry sodium sulfate, filtered and the solvent was distilled off from the filtrate.

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83,2 g of colourless or slight yellow oil was obtained and the fatty acid composition was identical with that obtained according to example 1.

#### Example 4

- 5            100 g of fatty tissue obtained according to example 2 was  
extracted with 800 ml of petroleum ether by homogenizing in the presence  
of 200 g of dry sodium sulfate. The tissue residues and salts were  
filtered off, the precipitate was washed with 2 x 100 ml of petroleum  
ether and the washings were combined with the extract. The solvent  
10        was distilled off in vacuo from the lipid solution thus obtained, 91 g of  
slightly yellow oil was obtained, the fatty acid composition being identical  
with that of the product obtained in example 2.

#### Example 5

- 15            During processing of one year old silver carp, fatty tissue  
was collected from the abdominal cavity. The material was stored in  
plastic container filled with ice and transported to the place of extraction  
at the temperature of melting ice.
- 20            5000 g of fatty tissue were placed into 20 l stainless container  
provided with Turrax stirrer, 5 l of water of 70 to 80 °C were added  
and the mixture was homogenized for 30 minutes. 2 x 500 ml of water  
were then added and after each addition the mixture was stirred for a  
few seconds. The equipment was cooled with water streamed through  
the cooling cap. After the temperature was decreased to 30 °C, the  
material was allowed to stand until an oily layer appeared on the surface  
25        of the aqueous suspension. The oil was removed by sucking with decanting  
pipe and the aqueous phase containing no oil was discarded. The oil  
was dried by filtering or separating thus obtaining 4650 g of slight  
yellow material.

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## Example 6

- 3 breams /Abramis brama/ weighing 250 g each were processed by removing the viscera from the abdominal cavity and the fatty tissues were collected. Thus, 30 g of fat tissue was obtained which was
- 5 crushed with blade homogenizer in the presence of 100 ml petroleum ether. The homogenizate was filtered and the filtrate was dried over dry sodium sulfate. The petroleum ether was distilled off in vacuo and an aliquot of the 25 g colourless fat sample thus obtained was methylated and the fatty acid composition was determined by gas chromatography.
- 10 The analysis data were compared with the triglyceride contents isolated from North Sea salmon /Salmon salar/.

	Fatty acid	Bream	Salmon
		Fat from abdominal cavity	Muscle triglyceride
15	14:0	6,6	6,6
	16:0	11,9	9,0
	18:0	0,5	0,2
	16:1 omega-9	12,5	6,3
	18:1 omega-9	20,6	21,3
20	20:1 omega-9	1,2	21,3
	22:1 omega-9	-	13,5
	18:2 omega-6	7,9	4,1
	20:2 omega-6	0,2	0,2
	20:3 omega-6	0,1	0,2
25	20:4 omega-6	5,3	0,6
	22:4 omega-6	0,6	-
	18:3 omega-3	7,3	1,0
	18:4 omega-3	2,8	2,7
	20:5 omega-3	0,6	0,6
30	20:5 omega-3	8,3	4,6

/cont./

22:5 omega-3	1,5	1,4
22:6 omega-3	5,4	7,2

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## Example 7

- 5 In the oil obtained according to any of the examples 3, 4 or 5 releasing of fatty acids was performed by dissolving the oil in 10-fold volume of petroleum ether and ethanolic potassium hydroxide in equivalent amount to the fatty acids present was added thereto. The system was allowed to stand for 10 to 16 hours at ambient temperature.
- 10 During this period saponification of the triglycerides took place. After cca. 12 hours equivalent amount of water was added to the reaction mixture, mixed and the layers were allowed to separate by allowing to stand or by separation. The upper petroleum ether layer was discarded while the lower layer contained the potassium salt of fatty acids which
- 15 were decomposed by acidifying to pH = 4,0 by the addition of hydrochloric acid, thus liberating the fatty acid. The free fatty acids were extracted from the aqueous solution into petroleum ether and then the solvent was distilled off, thus obtaining an oil.

## Example 8

- 20 From the fatty acids in the oil obtained according to any of examples 3, 4 and 5 methyl esters were formed by adding to the oil sodium methoxide dissolved in half volume of methanol. The sodium methoxide added was in molar equivalent with the fatty acid content. The reaction mixture was allowed to stand at room temperature for
- 25 10 to 16 hours and then a 10-fold volume of petroleum ether based on the fatty acids and water in an amount necessary for the separation of layers were added. The lower layer was discarded, the petroleum ether

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solution was washed with water and the solvent was distilled off.  
The oil thus obtained contained fatty acid methyl esters.

#### Example 9

5 The treatment of the free fatty acids obtained according to  
example 7 or the fatty acid methyl esters obtained according to  
example 8 for enriching in fatty acids of higher unsaturation grade  
was performed as follows:

From the oil materials 20 % methanolic solutions were  
prepared to which 20-fold quantity of urea dissolved in methanol based  
10 on the molecular weight of the fatty acids present was added. The  
quantity of methanol was chosen so that the fatty acid concentration  
of the final solution would be 10 %. The reaction mixture was allowed  
to stand at 3 to 5 °C for 24 hours. During this time the main amount  
of the saturated mono- and diene fatty acids and fatty acid methyl esters,  
15 respectively, formed an adduct with the urea which crystallized from  
the system. The crystals were filtered off under the same temperature,  
while cooling. The filtrate was diluted to double volume by water, the  
polyene fatty acids were extracted into petroleum ether and recovered  
by distilling off the solvent. Figures 3 and 4 show the urea purification  
20 of fatty acids obtained from fat tissues of silver carp by hot water  
extraction, saponification and liberation. Fig. 3 shows the fatty acid  
composition of the starting oil. Fig. 4 shows the analysis of the polyene-  
rich fatty acid mixture obtained by adduct forming according to the present  
example.

25 For preserving the fat tissue, if desired, deep freezing,  
or addition of antioxidants, e. g. 0,1 % of tokoferol or 0,05 % of butylat-  
ed hydroxytoluene can be used. The solvent extraction can be carried  
out e. g. in counter-current extractor while the aqueous extraction may  
be accomplished in autoclave by steaming the fat tissue.

The free fatty acids may also be liberated in buffered aqueous

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medium by enzymatic decomposition, e. g. with lipase.

The urea complex forming can be performed by heating at 50 to 70 °C for 30 to 60 minutes. To precipitate the adduct, the system is cooled below 30 °C.

- 5        The fatty acids can also be extracted from the aqueous solution thereof with other fat solvents, e. g. n-hexane, benzene, ethylacetate, etc. Before distilling off the solvent, those containing water, e. g. ethyl acetate, should be dried with sodium sulfate, resin treatment, etc.

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Claims

1. Process for preparing alimentation complementary composition containing omega-3 unsaturated fatty acids by extracting lipids from fishes, characterized in that as fish Hypophthalmichthis or bream /Abramis/ species are used and, if desired, the extract is purified by urea complex forming.
2. The process according to claim 1, characterized by using a Hypophthalmichthis species.
3. The process according to claim 2, characterized by using bred Hypophthalmichthis species.
4. The process according to any of claims 2 and 3, characterized by using silver carp or big head carp.
5. The process according to claim 1, characterized by using bream /Abramis brama/.
6. The process of any of claims 1 to 5, characterized by using the abdominal cavity fat of fishes as the starting material for extraction.

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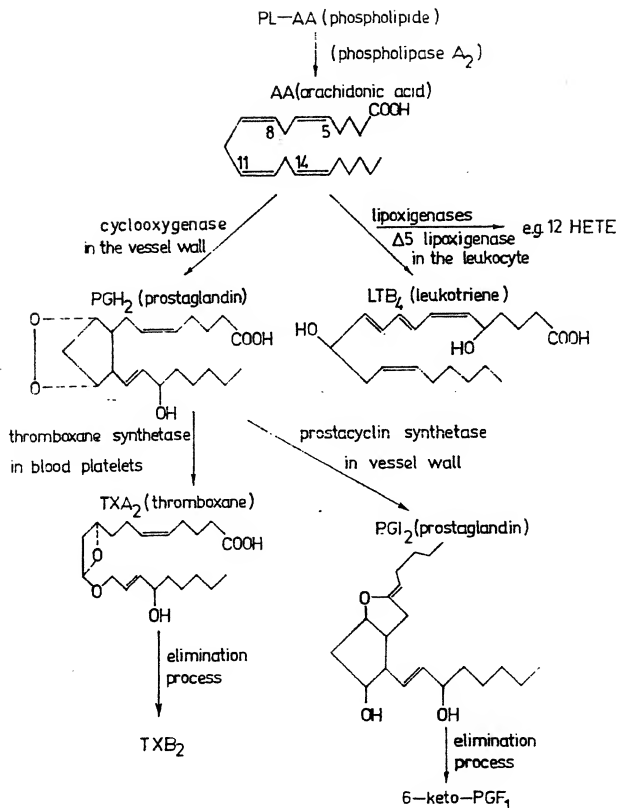


Fig.1

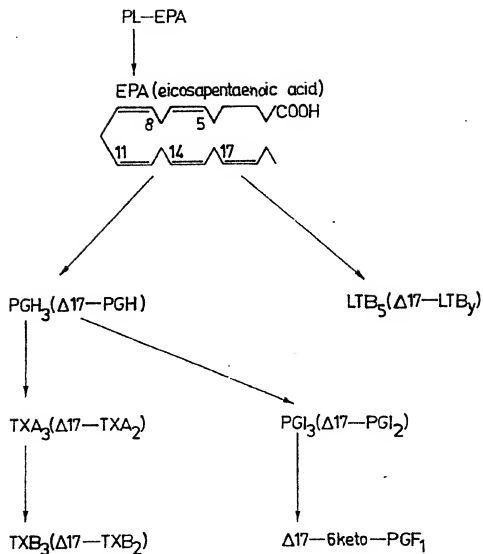


Fig.2

HPLC

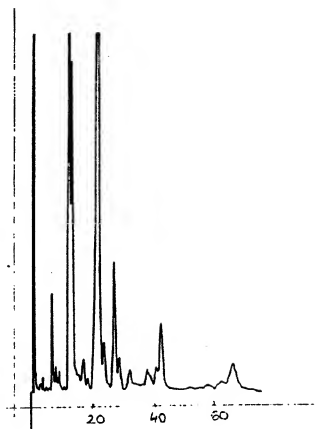


FIG. 3

HPLC

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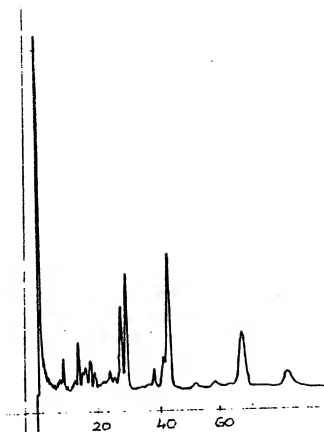


FIG. 4

# INTERNATIONAL SEARCH REPORT

International Application No PCT/HU 88/00027

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>4</sup> : C 11 B 1/10, A 23 L 1/327, A 23 D 5/00, A 61 K 35/60.	
<b>II. FIELDS SEARCHED</b> Minimum Documentation Searched *	
Classification System	Classification Symbols
Int.Cl. <sup>4</sup> : C 11 B 1/10; A 23 L 1/29, 1/30, 1/327; A 23 D 5/00; A 61 K 35/00, 35/56, 35/60.	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *	
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b>	
Category *	Citation of Document, <sup>11</sup> with Indication, where appropriate, of the relevant passages <sup>12</sup> Relevant to Claim No. <sup>13</sup>
Y	Chemical Abstracts, Volume 104, no. 6, issued 1986, February 10 (Columbus, Ohio, U.S.A.), S. Shigetoshi, "Extraction of therapeutic fatty acids from fish", see page 381, column 1, the abstract no. 39700u Jpn. Kokai Tokkyo Koho JP 60,170,700 (85,170,700). (1)
Y	Chemical Abstracts, Volume 101, no. 25, issued 1984, December 17 (Columbus, Ohio, U.S.A.), V.V. Kuzmina et al. "Features of fatty acid composition of lipids from fish intestinal mucosa in summer", see page 487, column II, the abstract no. 227279a Zh. Evol. Biokhim. Fiziol. 1984, 20(5), 542-5 (Russ). (1,5)
Y	Patent Abstracts of Japan, unexamined applications, C field, vol. 7, no. 66, March 18, 1983, The Patent Office Japanese Government, see page 64 C 157, Kokai No. 58-849 (Nippon Yushi K.K.). (1,6)
* Special categories of cited documents: <sup>14</sup> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document relating to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family	
<b>IV. CERTIFICATION</b>	
Date of the Actual Completion of the International Search 06 July 1988 (06.07.88)	Date of Mailing of this International Search Report 12 July 1988 (12.07.88)
International Searching Authority AUSTRIAN PATENT OFFICE	Signature of Authorized Officer <i>Pijpman</i>

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Patent Abstracts of Japan, unexamined applications, C field, vol. 8, no. 145, July 6, 1984, The Patent Office Japanese Government, see page 55 C 232, Kokai No. 59-51758 (Osame Kaisan K.K.).	(1)
A	US, A, 4 377 526 (T. FUJITA et al.), 22 March 1983 (22.03.83), see claims.	(1)
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Anhang zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

Annex to the International Search Report on International Patent Application No. PCT/HU 88/00027

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned International search report. The Austrian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Annexe au rapport de recherche internationale relatif à la demande de brevet international n°.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche internationale visé ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l'Office autrichien des brevets.

Im Recherchenbericht angeführtes Patent- dokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
JP-A2-58-000 849	06/01/83	None	
JP-A2-59-051 758	26/03/84	None	
US-A -4 377 526	22/03/83	JP-A2-57-187 397 JP-A2-58-008 037	18/11/82 18/01/83